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Selection of Crop Varities for Efficient Production Using Urea, Ammonia, Nitrate and Nitrite in the Closed Environment Life Support System

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Introduction: Crop production during extended space flights requires the development of procedures leading to the optimum use of the available energy. Since N assimilation is one of the most costly functions of a plant, optimizing its use represents an area where significant input could be made to the CELLS program. Since the oxidation of human waste and inedible plant parts can furnish a significant proportion of the N required to support the continuous growth of crops, a primary question is how the plants react to the expected products, nitrate, nitrite, ammonia, and urea alone and when mixed. Both wheat and barley were used in these studies, since both reacted similarly to the N sources.

Purpose of Study: To characterize and quantitate how the mixed N sources impose regulatory features at each point in the assimilatory pathways. To determine how these findings directly relate to overall N use efficiency.

Results. An overall view of N assimilation, e.g., nitrate, is represented by its absorption into the roots, wherein a portion is reduced, a portion translocated into the vacuole for storage, and a portion translocated into the shoot wherein the same allocations occur. The assimilatory pathways include net uptake (which includes influx and efflux), accumulation in the tissues, and reduction. Uptake includes induction and regulation of activity of the transport proteins. Accumulation includes determination of the storage sites and its relation to uptake and reduction. Reduction includes the regulation of the enzymes nitrate and nitrite reductases, their induction, turnover, and regulation of their activities. Each of these pathways has its own complex regulation Also included are the complex interactions of their integration with each other. Our results in each of these areas are described below.

Net Uptake. Net uptake is highly regulatory to all the pathways since it furnishes the initial flux of substrate. Each of the pathways react to the internal concentrations provided by uptake. Uptake in turn reacts to the external concentrations of the specific substrates. All of the uptake studies depended upon the development of an analytical system that allowed rapid, sensitive methods approaching constant analysis of the substrate solutions containing the four N compounds. We developed HPLC methods (Goyal, Huffaker, 1986a; Goyal et al. 1988;Thayer, Huffaker, 1980) to simultaneously assay for all four compounds in a nutrient solution in a mini growth chamber.

Characterization of nitrate and nitrite transporters. The nitrate and nitrite transporters were identified kinetically and the Km's determined (Aslam et al. 1992). A constitutive low Km system was found which absorbed both nitrate and nitrite (7 and 9 μM, respectively) (Aslam et al. 1992). In the presence of nitrate or nitrite, inducible transporters were found with higher Km's of about 35 and 45 μM, respectively for nitrate

and nitrite. A third constitutive linear nitrate and nitrite uptake system was detected at 40 μM and remains linear up through high concentrations. The low Km system allows the root to take up a small amount of nitrate required to induce the uptake and reduction systems. The plant has developed several mechanisms for taking up inorganic N allowing it to react to a wide range of concentrations. Nitrate and nitrite are superb competitive inhibitors of each others uptake (Aslam et al. 1992). This must be taken into account when both are present in a nutrient solution.

Is NR required for nitrate uptake? NR has been proposed to play a role in the uptake of nitrate. This study conclusively showed that nitrate uptake occurred in mutant barley plants lacking both NAD and NADH specific nitrate reductases (Warner, Huffaker, 1989). The mutant plants absorbed as much nitrate as the normal plants and accumulated the nitrate in place of reducing it.

Interactive effects of nitrate, nitrite, ammonium, and urea on uptake. Net uptake rates of wheat seedlings from substrate solutions containing all four compound were monitored simultaneously (Criddle et al. 1988). Although urea uptake was too slow to monitor, its presence had major inhibitory effects on the uptake of the other compounds. Rates of nitrate, ammonium, and nitrite uptake depended in a complex fashion on the concentration of all four N compounds. Equations were developed which describe the uptake rates of each of the compounds, and of total N, as functions of concentrations of all N sources. This work represents a solid approach to optimize the use of the four N sources.

Nitrate and nitrite efflux. Evidence now shows that efflux represents a serious loss of nitrate from the roots during the uptake process as the external concentration increases. At 1mM nitrate, about half of the nitrate influxed is effluxed. Since many of the investigators in the CELSS program use very high concentrations of nitrate in their nutrient solutions, efflux could greatly decrease efficient use of nitrate. We considered the study of efflux to be one of the most critical issues studied during the tenure of the grant. Some unique methods were developed for this study (Aslam et al. 1994). The rate of efflux was dependent upon the concentration of nitrate or nitrite in the cytoplasm in the root cells. In turn the concentration in the cytoplasm was dependent upon the rates of uptake, translocation into the vacuoles, and reduction. At low external concentrations of substrates, efflux was much less serious and N use efficiency was much greater.

Effect of ammonium on efflux. Prior to this work, it was thought that ammonium inhibited net uptake of nitrate by inhibiting influx. We showed that ammonium inhibits net nitrate uptake by increasing efflux; ammonium did not affect influx (Aslam et al. 1994). Ammonium increased efflux in direct proportional to the internal concentration of nitrate. Since most nutrient solutions contain both nitrate and ammonium, N use efficiency decreases as the concentration of nitrate increases.

Effect of pH and Ca^{2+} on influx and efflux. At acidic pH, the decrease in net nitrate uptake is due to the stimulation of efflux with no effect on influx, whereas at basic pH, it

is due to the inhibition of influx. At acidic pH, Ca^{2+} increased net uptake by decreasing nitrate efflux (Aslam et al. 1995).

Root perturbation and excision on nitrate influx and efflux. Investigators have often worried that perturbation of the roots during uptake experiments may affect the uptake rates of an ion. In this study, perturbation was simulated by mechanically striking the intact roots with a glass rod. The results showed that root perturbation and excision had no effect on nitrate influx but inhibited net uptake by stimulating efflux (Aslam et al., 1996).

Induction of uptake systems. The high Km nitrate and nitrite transport systems were induced linearly up to 10 μM by either nitrate or nitrite after a short lag period (Aslam et al. 1993; Goyal, Huffaker, 1986b). Above 10 μM nitrate, induction by nitrate continued more slowly, induction by nitrite decreased sharply, apparently due to nitrite toxicity (Aslam et al. 1993). Ammonium partially inhibited both the nitrate and nitrite transporters. The nitrate transporter was twice as sensitive to ammonium than the nitrite transporter (Aslam et al. 1996). . This may relate to higher turnover rates of membrane-associated nitrate transport proteins

Reduction and assimilation. Nitrate and nitrite are reduced to the level of ammonium by the enzymes nitrate reductase (NR) and nitrite reductase (NiR). Ammonium is assimilated via glutamate synthase and glutamate synthetase. Urea is converted to ammonium and CO_2 by urease; ammonium is then assimilated in the usual way. Both NR and NiR are induced by nitrate and are in constant turnover (e.g., Velasco et al 1989).

Induction of NR and NiR (leaves). The comparative induction of NR and NiR by ambient nitrate and nitrite as a function of influx, reduction (as NR was induced) and accumulation in leaves was determined (Aslam et al. 1987). A dynamic interaction amongst these processes was found. The activity of NR, as it was induced, influenced its further induction by affecting the internal concentration of nitrate. As the ambient concentration of nitrate increased, the relative influences imposed by influx and reduction on nitrate accumulation changed with influx becoming a more predominant regulant. Significant concentrations of nitrate accumulated in nitrite-fed leaves. NR was not induced by nitrite until nitrate appeared in the leaves. Nitrate was the more likely inducer of NR than was nitrite. Evidence showed that NiR was induced by nitrate directly, without being reduced to nitrite. Absorbed nitrite induced NiR indirectly after being oxidized to nitrate within the leaf (Aslam, Huffaker, 1989).

Induction in roots. Although both ions induced NR NR was effective at a lower concentration than was nitrite (Aslam et al. 1993). Both ions equally induced NiR.

Possible Relationship of NR and the nitrate transport protein. Membrane associated NR was detected in plasma membrane (PM) fractions isolated from barley roots. Anti-NR immunoglobulin G fragments purified from anti-NR serum inhibited nitrate uptake by more than 90% but had no effect on nitrite uptake (Ward et al. 1988, 89). It is

possible that these two PM proteins may be related. It also indicates that the nitrite transporter may be distinct from the nitrate transporter.

Experimental significance of the studies..The study represented an effective use of basic approaches leading to a practical understanding of how to effectively use a combination of nitrate, nitrite, ammonium, and urea in a nutrient solution with an eye towards N use efficiency. Since these are the expected products of recovery from biological materials, it seems important to reutilize them with a minimum of chemical conversions. We provided a basis for understanding their respective uptake and assimilation along with the interactions in the assimilation processes. We further provided a method and equations to calculate the optimum concentrations for uptake of the mixed sources.

Expression of appreciation. We enjoyed dealing with the people in the CELSS program and especially our interactions with Dr. Robert McElroy at the Ames Research Station. We appreciated the support which significantly helped our university research program. In addition, we feel satisfied that our contributions added to the progress of the CELSS program.

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